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Ryegrass Cultivars and Endophyte in Tall Fescue Affect Nematodes in Grass and Succeeding Soybean

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ABSTRACT

Annual ryegrass (*Lolium multiflorum* Lam.) is often double cropped with soybean [*Glycine max* (L.) Merr.] in much of the southeastern United States to provide winter grazing and ground cover. Little is known about the effect of annual ryegrass cultivars on nematodes associated with the grass, and with subsequent soybean crops. In several other taxonomically similar grasses, including tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.), the presence of endophytic fungi imparts pest resistance to the grass. No information is available on the effect of these endophytic fungi on nematodes associated with the grass. Therefore, the objectives of this study were to determine: (i) the effect of annual ryegrass cultivars on nematodes in the grass and in succeeding soybean, and (ii) the effect of *Acremonium coenophialum* Morgan-Jones and Gams in tall fescue on nematodes. Thirteen annual ryegrass cultivars, 'Kentucky 31" tall fescue with and without *A. coenophialum* infection, and a nematode-susceptible soybean check were grown for 7 wk in pots in the greenhouse in a Malibis sandy loam (fine-loamy, siliceous, thermic Plinthic Palendults) soil known to be infested with high numbers of phytonematodes. Plants were then removed from the soil, and the soybean was seeded into the pots and grown for 7 wk. Significant differences among nematode populations in the annual ryegrass cultivars occurred, and the nematode numbers were generally higher in the soybean that followed. However, these increased numbers did not affect soybean performance. Therefore, it does not appear that the use of annual ryegrass–soybean doublecropping systems will be effective in controlling most nematodes when using nematode-susceptible soybean cultivars. In tall fescue, lower spiral [*Helicotylenchus dihystera* (cobb) Sher.] and stubby root [*Paratrichodorus minor* (culbran) Siddiqi] nematode numbers were associated with *A. coenophialum* in the grass. Since nematode damage has previously been associated with stand decline in tall fescue, breeders working in nematode-infested areas should be aware that decreased pest resistance may be associated with the removal of *A. coenophialum* from tall fescue.

ANNUAL RYEGRASS is grown as a winter forage crop or ground cover in rotation with soybean in much of the southeastern United States. Such doublecropping with an annual grass is thought to reduce the plant parasitic nematode populations in the succeeding soybean crop. However, recent research has demonstrated that nematode reduction does not necessarily occur in rye (*Secale cereale* L.)-peanut (*Arachis hypogaea* L.) or in corn (*Zea mays* L.)-peanut rotations (Rodriguez-Kabana and Ivey, 1986). Rodriguez-Kabana and Touchton (1984) showed that corn and sorghum [*Sorghum bicolor* (L.) Moench] rotation systems with peanut did reduce initial nematode populations in the soil, but that the populations quickly recovered in the peanut.

Cultivar differences are known for nematode response in soybean (Rodriguez-Kabana and Thurlow, 1980; Rodriguez-Kabana and Weaver, 1984; Weaver et al. 1985). However, differences in nematode response to annual ryegrass cultivars per se, or in a double cropping system with soybean, have not been described. Variable cultivar response in the grass could impact the following soybean crop.

Tall fescue, perennial ryegrass, and numerous other grasses host endophytic fungi that are detrimental to herbivorous organisms feeding on the grass (Bacon et al., 1986). Little is known about the effect of these endophytic fungi on plant parasitic nematodes attacking the grasses. Nematodes have been implicated in stand decline in tall fescue (Hoveland et al., 1975, 1978), but improved persistence of tall fescue and perennial ryegrass has been attributed to the presence of endophytic fungi (Hurley et al., 1984). It is possible that nematode response to grasses may be modified by the presence of these endophytic fungi.

The objectives of this study were to determine: (i) the effect of annual ryegrass cultivar on nematode

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populations associated with the grass and in succeeding soybean, and (ii) the effect of *Acremonium coenophialum* Morgan-Jones and Gams in tall fescue on nematodes.

MATERIALS AND METHODS

The following annual ryegrass cultivars and lines (hereafter simply referred to as cultivars) were used: 'Bison', 'Florida 80', 'Gulf', 'Marshal', 'Ninak', 'Penploid-4', Pioneer 5M5F, Pioneer 7F3M, PS-1005 (from Pacific Seed Production Co., Albany, OR), 'Shannon', 'Tetrablend-44', 'Urbana', and 'Van Hi 77'. Seed were examined microscopically to determine endophyte infection levels, using the method described by Clark et al. (1983). The number of seeds examined and the infection level were recorded for each seed lot. Two seed lots of 'Kentucky 31' tall fescue having known *A. coenophialum* infection levels of 0 and 98% were used. A nematode-susceptible soybean ('Davis') was included as a check.

All entries were seeded in 100-mm-diam. plastic pots (1-L capacity) in the greenhouse on 13 Feb. 1985. The grasses were seeded at a rate of 0.5 g per pot, and the soybean check was seeded at five seeds per pot and hand thinned to four plants per pot after emergence. The soil was a Malbis sandy loam infested with spiral, stubby root, and root-knot [*Meloidogyne incognita* (Kofoid and White) Chitwood] nematodes at levels of 10, 15, and 50 adults or juveniles per 100 mL soil, respectively. The pots were surface watered daily and were well drained. Ambient temperature was maintained at 23 to 28°C during the experiment. After 7 wk, the plants were removed from the soil by carefully shaking the soil from the roots. Total number of plants, total fresh shoot weights, and total fresh root weights were recorded. Nematodes in the remaining soil and root fractions were counted using the method described by Rodriguez-Kabana and Pope (1981).

Davis soybean was seeded in all the pots after grass removal on 7 May 1985. Seeding rates and cultural treatment of the soybean were as described above. On 21 June 1985, the soybean was harvested, and data were recorded as described above for the grasses.

The treatments were replicated 10 times in a randomized complete–block design. Data were analyzed using analysis of variance, and mean comparisons were made using the Waller-Duncan multiple range test when treatment differences were significant. Single degree-of-freedom comparisons were made for Kentucky 31 tall fescue at the two levels of *A. coenophialum* infection.

Table 1. Endophyte infection rates in seeds of 13 annual ryegrass cultivars.

Cultivar	Number of seeds examined	Percent infection
Bison	31	0
Florida 80	30	3
Gulf	32	3
Marshal	30	43
Ninak	50	0
Penploid-4	30	0
Pioneer-5M5F	30	3
Pioneer-7F3M	30	13
PS-1005	30	0
Shannon	36	0
Tetrablend-444	30	0
Urbana	50	0
Van hi 77	31	0

RESULTS AND DISCUSSION

Marshal was the only annual ryegrass cultivar in which a substantial percentage of the seed was infected with an endophyte (Table 1). In screening tests, the fungus closely resembled the endophytes known to infect tall fescue and perennial ryegrass. Identification of this fungus and determination of its significance in annual ryegrass will require further study.

Spiral, stubby root, and root knot were the major nematodes present in the pots after the ryegrass had grown for 7 wk. Spiral nematodes are ecto/endopar-

Table 2. Nematode populations associated with 13 annual ryegrass cultivars and tall fescue with and without *Acremonium coenophialum* infection.

Cultivar	Spiral		Stubby		Root Knot	
	Root	Soil	Root	Soil	Soil	Gall
	no./5 g	no./100 cm³	no./5 g	no./100 cm³	no./5 g	no./5 g root
			Annual ryegrass			
Bison	5b†	34abc	32c	197a	0c	3b
Florida 80	6b	25abcde	17c	61d	14b	2b
Gulf	6b	24bcde	18c	95cd	2c	5b
Marshal	6b	27abcd	32c	161ab	4c	10b
Ninak	13b	10de	72b	190a	0c	8b
Penploid-4	12b	31abc	34bc	185a	6c	9b
Pioneer-5M5F	6b	24abcde	39bc	170ab	4c	3b
Pioneer-7F3M	4b	42ab	42bc	96cd	0c	9b
PS-1005	14b	31abc	41bc	154ab	1c	9b
Shannon	5b	7e	36bc	109c	1c	3b
Tetrablend-444	10b	37ab	23c	138bc	2c	9b
Urbana	6b	43a	17c	192a	1c	2b
Van hi 77	10b	25abcde	16c	176ab	8bc	9b
			Soybean			
Davis (control)	152a	16cde	170a	1e	69a	171a
			Tall fescue			
KY 31 (0%)‡	20	19§	120¶	106	1	0
KY 31 (98%)	6	3	30	94	0	0

† Numbers in a column followed by the same letter are not significantly different at $P = 0.05$ using Duncan's multiple range test.
‡ KY 31 (0%) = Kentucky 31 tall fescue with 0% *A. coenophialum* infection, and KY 31 (98%) = Kentucky 31 tall fescue with 98% *A. coenophialum* infection.
§ KY 31 (0%) is significantly different than KY 31 (98%) at the 0.07 level of probability using single-degree-of-freedom comparisons.
¶ KY 31 (0%) is significantly different than KY 31 (98%) at the 0.05 level of probability using single-degree-of-freedom comparisons.

asites, so both soil and root samples are good indicators of response to cultivar. Stubby root nematodes are predominately ectoparasites, so the soil samples should normally be the better indicator of response to cultivar.

Annual ryegrass cultivars affected nematode populations associated with the grass (Table 2). Shannon and Ninak had particularly low numbers of spiral nematodes present in the soil after 7 wk. No single cultivar showed extreme susceptibility to spiral nematodes when compared to the soybean roots, indicating that ryegrass is not a good host for *H. dihystera*.

Florida 80 showed low numbers of stubby root nematodes in both root and soil samples. However, differences due to ryegrass cultivar effects on stubby root nematodes are not clearly apparent. Disagreement between root samples and soil samples make inferences difficult. For instance, Urbana had low stubby root numbers on root samples, but high numbers in soil samples. Relatively high numbers of nematodes were found on the root samples of the soybean check when compared to the soil samples, indicating incomplete separation of the parasite from the root when the soil and roots were separated.

Although annual ryegrass has not previously been described as a host of root knot nematodes, several cultivars showed evidence of reproduction and galling. florida 80 and Van hi 77 had the highest associated reproduction, as evidenced by numbers of root knot nematodes in the soil. This would appear likely to impact the nematode numbers and damage to the plant in succeeding soybean crops, but no clear relationship was seen in either nematode numbers (Table 3) or plant parameters in soybean following these ryegrass cultivars.

Spiral nematode numbers were lower in soil fractions from tall fescue infected with *A. coenophialum* than in soil fractions from the *A. coenophialum*-free tall fescue (Table 2). Although the mean value for numbers of spiral nematodes in the *A. coenophialum*-free tall fescue root fractions was threefold that of the *A. coenophialum*-infected tall fescue root fractions, they were significantly different at only the 0.28 level of probability.

A significant difference in stubby root nematode numbers between *A. coenophialum*-infected and *A. coenophialum*-free tall fescue was observed only in the root fractions. Although stubby root nematodes are usually ectoparasites, many of the stubby root nematodes probably adhered to the roots when the root and soil fractions were divided.

As expected, root knot nematodes did not reproduce well on either of the tall fescues. No galling of roots was noted, and only negligible occurrences of the nematodes themselves were recorded in the soil samples.

The number of plants per pot, shoot weight, and root weights were all higher for the *A. coenophialum*-infected tall fescue than the *A. coenophialum*-free tall fescue. Germination tests were run on both tall fescue seed lots prior to the experiment. Both had 85% germination. The number of plants per pot was three times higher (P=0.05) for the *A. coenophialum*-infected tall fescue than the *A. coenophialum*-free tall fescue (78 vs. 26) at the end of 7 wk. Shoot weights and root weights of the *A. coenophialum*-infected tall fescue were roughly double (P=0.05) those of the *A. coenophialum*-free tall fescue (5.2 vs. 3.2 g, and 9.9 vs. 4.6 g, respectively). This clearly indicates that the *A. coenophialum*-infected tall fescue grew better under these conditions. In an earlier study, Pedersen and Rodriguez-Kabana (1984) concluded that spiral and stubby root nematodes were potentially the most important nematodes on tall fescue in the southeastern United States. It is therefore especially important to recognize that these same nematodes show increased

Table 3. Nematode numbers associated with soybean following 13 annual ryegrass cultivars and tall fescue with and without *Acremonium coenophialum* infection.

Cultivar	Spiral		Stubby	Cyst			Root knot		
	Root	Soil	Soil	Root	Soil	Gall	Root	Soil	Gall
	no./5 g	no./100 cm³	no./100 cm³	no./5 g	no./100 cm³	no./5 g root	no./5 g	no./100 cm³	no./5 g root
				Previous crop—annual ryegrass					
Bison	17d†	21cde	97ab	17a	24bc	2a	45d	3abc	18ab
Florida 80	50ab	17def	46de	42a	23bc	1a	91d	0	23ab
Gulf	39abcd	35b	38ef	23a	22bc	4a	123bcd	4abc	14ab
Marshal	41abcd	6f	54de	25a	9c	2a	57d	0c	23ab
Ninak	43abc	31bcd	104a	29a	36ab	1a	129bcd	5abc	11ab
Penploid-4	20cd	56a	64cde	14a	25bc	1a	60d	0c	18ab
Pioneer-5M5F	21cd	8ef	56de	9a	17bc	0a	99cd	5abc	16ab
Pioneer-7F3M	48ab	19cdef	59cde	25a	22bc	0a	115bcd	0c	18ab
PS-1005	43abc	58a	81abcd	25a	25bc	1a	111bcd	2bc	16b
Shannon	31bcd	18def	72abcde	32a	32abc	2a	128bcd	6ab	19ab
Tetrablend-444	63a	68a	100abcde	21a	30bc	3a	271a	7a	23ab
Urbana	35bcd	32bc	76abcd	44a	36ab	1a	189ab	2abc	31a
Van hi 77	50ab	30bcd	95abc	37a	23bc	0a	184bc	7a	22ab
				Previous crop—soybean					
Davis (control)	33bcd	19bcd	9f	42a	44a	1a	57d	1c	6b
				Previous crop—tall fescue					
KY 31 (0%)‡	2	4	49	15	30	0	8	2	2*
KY 31 (98%)	15	12	52	45	21	2	1	0	13

* KY 31 (0%) is significantly different than KY 31 (98%) at the 0.05 level of probability using single-degree-of-freedom comparisons.
† Numbers in a column followed by the same letter are not significantly different at P = 0.05 using Duncan's multiple range test.
‡ KY 31 (0%) = Kentucky 31 tall fescue with 0% *A. coenophialum* infection, and KY 31 (98%) = Kentucky 31 tall fescue with 98% *A. coenophialum* infection.

reproduction in pots seeded to *A. coenophialum*-free compared to pots seeded with *A. coenophialum*-infected seed. Producing *A. coenophialum*-free cultivars of tall fescue may well lead to decreased pest resistance by this species.

Annual ryegrass cultivars affected the number of nematodes in soybean following the annual ryegrass (Table 3). However, no one cultivar was consistently associated with high or low values for all the nematode parameters measured in the soybean. The most striking observation was that nematode counts and galling in soybean following the nematode-susceptible soybean check were among the lowest values for all parameters measured, except cyst (*Heterodera glycines* Ichinohe) nematodes in the soil. This leads us to suspect that the use of one season of annual ryegrass to control nematodes in soybean doublecropping systems will be ineffective. Even in the case of cyst nematodes, or where a particular annual ryegrass cultivar suppressed a particular target nematode, we would expect the population dynamics of the nematodes to quickly compensate for initially lower nematode numbers (Rodriguez-Kabana and Touchton, 1984).

Soybean performance, as measured by fresh shoot and root weight after 7 wk of growth, was not affected by annual ryegrass cultivar. Average soybean top weight following ryegrass was 4.1 g and average soybean root weight was 2.4 g. The soybean following the soybean check yielded more roots in all cases (4.6 g), and gave equivalent shoot yields (5.4 g) when compared to soybean following annual ryegrass ($P=0.05$). However, in the field, a recent study has shown increased yields of soybean following a rye winter cover crop without a reduction in final nematode populations (Rodriguez-Kabana and Ivey, 1986).

Soybean following *A. coenophialum*-free tall fescue had lower shoot weights ($P=0.05$) than soybean following *A. coenophialum*-infected tall fescue (3.9 vs. 7.4 g). Since no differences were found in nematode numbers in soybean following either of the tall fescues, differences in soybean performance would not appear to be directly related to nematode supression by the *A. coenophialum*-infected tall fescue.

Differences in nematode reaction to annual ryegrass cultivars do exist. However, in annual ryegrass, as in other crops, nematode resistance or susceptibility will probably be specific for individual species. Annual ryegrass cultivars planted prior to soybean did affect the nematode numbers on the soybean. But, with the exception of cyst nematodes, no annual ryegrass cultivar was associated with lower nematode numbers in soybean following the annual ryegrass than in soybean following a nematode-susceptible soybean check. Therefore, it does not appear that the use of annual ryegrass-soybean doublecropping systems will be effective in controlling most nematodes when using nematode-susceptible soybean cultivars. In practice, this would not negate other values of such a doublecropping system. Advantages, such as increased total production per year and erosion control, continue to make doublecropping systems attractive management tools.

Acremonium coenophialum infection in tall fescue does impart some degree of nematode resistance to tall fescue. Since nematode damage has previously been associated with stand decline in tall fescue, breeders working in nematode-infested areas should be aware that decreased pest resistance may be associated with the removal of *A. coenophialum* from tall fescue.

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